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Orientation of the Guanine Operon of *Escherichia coli* K-12 by Utilizing Strains Containing *guaB-xse* and *guaB-upp* Deletions

LYNNE D. VALES, JOHN W. CHASE,* AND JANET B. MURPHY

Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York 10461

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Temperature induction of an *Escherichia coli* strain with λcI857 integrated in the *guaB* gene has been used to produce strains containing chromosomal deletions extending into the *xse* and *upp* genes. By utilizing strains containing these deletions, it has been possible to order the genes in the guanine operon with respect to the *xseA* and *upp* genes. The order of the genes in this region is *glyA-hisS-xseA-guaO-guaB-guaA-purG-upp-purC*.

To extend our studies of the in vivo and in vitro properties of *Escherichia coli* exonuclease VII, we have isolated λ transducing particles carrying the gene(s) for the enzyme. In the course of this work, we have isolated various deletion mutants, the analysis of which has enabled us to determine the orientation of the guanine operon as well as the position of the genes controling the production of exonuclease VII relative to this operon. The guanine operon of *E. coli* has been mapped at approximately 53 min on the recalibrated *E. coli* genetic map (1). The operon comprises genes involved in the regulation and synthesis of GMP (6, 7). The order of the genes in the operon has been shown to be *guaO-guaB-guaA* (4). However, its orientation with respect to nearby loci was unknown. A strain of *E. coli*, KS504, has been isolated by Shimada et al. (9, 10) in which λcI857 is integrated in the *guaB* gene. In addition, they have determined the orientation of the integrated phage (9). Temperature induction of this lysogen allows isolation of strains with chromosomal deletions extending from *guaB* to genes on either side of *guaB*. *xseA* is a structural gene for the single-strand-specific DNase exonuclease VII (2) and is 94% cotransducible with *guaA* (3). It was not known on which side of the guanine operon *xseA* is located. The gene coding for UMP pyrophosphorylase, *upp*, had been tentatively mapped at a position on the chromosome between *purC* and the guanine operon (8). Derivatives of strain KS504 provided donors for use in P1 transduction studies which have established the genotype of these deletion mutants and thus the orientation of the guanine operon with respect to *xse* and *upp*. In addition, studies by Parker and Fishman (7a) have indicated that *purG* was incorrectly placed on the *E. coli* linkage map of Backmann et al. (1), and our studies with a *guaB-upp* deletion mutant have confirmed their results.

The strains used and their genotypes are given in Table 1. P1 transductions were performed as described by Miller (5). Strains containing deletions in *guaB* were isolated by plating 0.1 ml of a log-phase culture of KS504 grown in L-broth (5) at 30°C onto tryptone plates (5) and incubating at 43°C. Colonies were picked to 96-well microtiter plates containing 100 μl of L-broth per well and incubated at 43°C. Cultures were checked for guanine requirements by replica plating onto Vogel Bonner minimal plates (11) with 10 μg of biotin per ml. Temperature-resistant strains exhibiting a Gua^- phenotype were assayed for exonuclease VII activity (3). To obtain strains with deletions extending from *guaB* to *upp*, derivatives of KS504 were selected for 6-azauracil resistance at 43°C. Cells were grown to log phase, centrifuged, and resuspended in 0.85% NaCl with a 10-fold concentration of cells. Cells (0.1 ml) were spread onto Vogel-Bonner plates containing 10 μg of biotin per ml, 20 μg of GMP per ml, and 50 μg of 6-azauracil per ml and incubated at 43°C. Nutritional tests to identify deletions in the guanine operon were performed according to Shimada et al. (9). Sensitivity to λcI857 infection was determined by the spot test (5).

From a total of 2×10^9 cells of strain KS504, temperature-resistant colonies at 43°C were obtained at a frequency of 2×10^{-6}. Approximately 4% required guanine for growth and were assayed for exonuclease VII activity (3). Four of these contained no detectable exonuclease VII activity (Table 2). Three of these four strains were sensitive to λcI857, indicating that most of the prophage genome including the immunity region had been deleted. One of the exonuclease VII-deficient strains (KLC380) was resistant to λcI857 but was sensitive to λ*vir*, suggesting that only a portion of the prophage genome had been deleted and that the immunity region remained. These results suggest that deletion of the prophage genome resulted in at least a partial deletion of the gene(s) involved in the production of exonuclease VII. All four exonuclease VII-deficient strains exhibited phenotypes, suggesting that they were *guaB*, *guaA*$^+$, and *upp*$^+$ (Table 2). P1 transduction studies with these strains as donors (Table 2) indicated that the strains contained intact *guaA* and *purG* genes but were defective in the *xseA* gene. Therefore, the *xseA* gene must be on the operator side of the guanine operon. Although these studies cannot order *xseA* and *guaO*, we have tentatively placed *guaO* between *xseA* and *guaB* (Fig. 1).

Derivatives of KS504 exhibiting 6-azauracil and temperature resistance were obtained at a frequency of 5×10^{-8}. Of 34 strains isolated by this selection, all were unable to grow on minimal plates supplemented with xanthine but could grow with guanine, suggesting that they were defective in both the *guaA* and *guaB* genes (Table 2). All exhibited wild-type levels of exonuclease VII activity. All strains tested were found to be sensitive to λcI857. One of these strains, KLC421, was used as a donor in P1 transduction studies (Table 2) and was shown to be defective in the *guaA* and *purG* genes. The deletions in these strains, therefore, are in the direction opposite to that which gave deletions in the *xse* region and extend from *guaB* through *guaA* and into the *purG* and *upp* genes (Fig. 1). These studies confirm the results of Parker and Fishman (7a) that *purG* had previously been incorrectly placed in the region between *glyA* and the guanine operon (1) and correctly place it in the region between the guanine operon and *dapE*.

The *xseA*, *guaB*, and *guaA* genes have been ordered with respect to nearby genetic loci, thereby establishing the orientation of the guanine operon. The extent of the *xseA*-*guaB* and *guaB*-*upp* deletions which we have isolated has not been accurately determined. However, Parker and Fishman (7a) have mapped *hisS*, an essential gene coding for histidyl tRNA synthetase, at 0.15 min from *guaA* on the *glyA* side of

TABLE 1. *Bacterial strains*

Strain	Relevant genotype	Source
PCO568	*guaA*	De Haan
PCO631	*purG48*	*E. coli* genetic stock center strain 4497
KLC19	*purC50*	Chase
KLC375	*glyA6*	Chase
KLC382	*xseA7 glyA6*	Chase
KS504	HfrH Δ(*gal-bio*) λcI857 within *guaB*, single lysogen[a]	Shimada
KLC380	Δ(*guaB-xse*)	This work, from KS504
KLC381	Δ(*guaB-xse*)	This work, from KS504
KLC405	Δ(*guaB-xse*)	This work, from KS504
KLC410	Δ(*guaB-xse*)	This work, from KS504
KLC421	Δ(*guaB-upp*)	This work, from KS504

[a] Δ denotes a deletion.

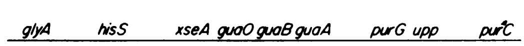

FIG. 1. *Genetic map of E. coli K-12 in the region between glyA and purC. The location of hisS and the order of purG and upp is taken from Parker and Fishman (7a).*

TABLE 2. *Properties of KS504 derivatives containing deletions from the guaB gene*

Strain	Exonuclease VII activity	Sensitivity to 6-azauracil	Growth on XMP[a]	Sensitivity to λcI857[b]	Genotype of *gua* region[c]				
					glyA	*xseA*	*guaA*	*purG*	*purC*
KLC380	−	+	+	−	+	−	+	+	+
KLC381	−	+	+	+	+	−	+	+	+
KLC405	−	+	+	+					
KLC410	−	+	+	+					
KLC421	+	−	−	+			−	−	+

[a] Growth on XMP indicates a functional *guaA* gene product.

[b] Strain KLC380 was sensitive to λ*vir*, indicating that it was not λ resistant.

[c] P1 transductions were performed with P1CM*clr*100 grown on the strains in the first column. The recipients were: KLC375 (*glyA*); KLC382 (*xseA*); PCO568 (*guaA*); PCO631 (*purG*); KLC19 (*purC*); blanks indicate genes not tested.

the guanine operon. We conclude that the deletions in the chromosome resulting in *xse-guaB* deletion mutants must encompass less than 0.15 min. It is clear that the region between *xseA* and *upp* cannot contain any essential genes and therefore exonuclease VII as well as UMP pyrophosphorylase cannot be essential for cell survival. Further studies of strains lacking exonuclease VII activity in conjunction with λ transducing particles derived from KS504 are in progress to analyze the genetic aspects of the production and possible control of exonuclease VII.

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